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Water balance response in the terrestrial Western Australian frog, *Myobatrachus gouldii* (Anura: Myobatrachidae)

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Abstract

Dehydrated turtle frogs (*Myobatrachus gouldii*) slowly rehydrate in distilled water, reaching initial body mass after 160 min and 114% of initial mass after 450 min. The instantaneous rate of rehydration declined from the initial rate of 116 mg g^{-1} h^{-1} to 10 mg g^{-1} h^{-1} after 450 min. The turtle frog has the lowest rehydration rate measured from 16 Western Australian frogs, presumably reflecting the rarity of free water in its natural habitat and absence of a selective pressure for a marked water balance response. It has a large bladder volume, about 20% of body mass, and its urine is considerably more dilute than blood.

Introduction

The life history of most terrestrial amphibians is linked by their reproductive requirements to the presence of free-standing water. Almost invariably, eggs, tadpoles, or both stages require water for completion of development. Not surprisingly, iono- and osmoregulation by amphibians is controlled primarily by hydration across the skin from free-standing water, although many amphibians can absorb water from moist soil (Packer 1963, Bentley 1971, Bentley & Main 1972, McClanahan 1972, Shoemaker *et al.* 1992). The water balance response (WBR or Brunn effect) of amphibians is the restoration of body mass by the cutaneous reabsorption of water, primarily across the highly vascular pelvic skin under hormonal control by the posterior pituitary hormone, arginine vasotocin (AVT; Ewer 1952, Shoemaker & Waring 1968, Bentley 1971, Shoemaker *et al.* 1992). However, reproduction by some amphibians does not require free water; their eggs directly develop into froglets, without a free-swimming tadpole stage (Bogart 1981, Duellman & Trueb 1986). This reproductive strategy is used by two Australian frogs, the turtle frog *Myobatrachus gouldii* and *Arenophryne rotunda*, which deposit their eggs up to 1 to 2 metres underground (Roberts 1981, Roberts 1984).

Myobatrachus gouldii occurs in the south-western region of Western Australia, primarily in sandy soils of the Wandoo belt but not the forest regions (Tyler, Smith & Johnstone 1984). It is capable of surviving without free-standing water (How & Dell 1989). Individual turtle frogs might spend their entire life without ever encountering free-standing water, so iono- and osmoregulation by these frogs must usually occur in the absence of free-standing water. Consequently, they might be expected to have a poor water balance response.

This study investigates the water balance response of turtle frogs, and determines whether it is lower than for other frogs and whether turtle frogs are able to maintain ionic and osmotic equilibrium in free-standing water.

Methods

Turtle frogs were collected from sandy sites east of Two Rocks, 55 km north of Perth, in November 1986 and returned to the laboratory in moist sand (approximately 1% water content) from the collection locality. All experiments were conducted as soon as possible after return to the laboratory.

Normally-hydrated individuals were washed free of sand, dried with paper towelling, and induced to urinate by inserting a polished glass cannula into the cloaca; the subsequent standard body mass was measured to ± 0.001 g with a Sauter RL200 balance. The animals were then dehydrated overnight in dry containers by exposure to laboratory conditions. Body mass was reduced to approximately 80% of the standard mass. Individuals were then placed in a container with approximately 3 mm depth of distilled water, and reweighed at intervals, for up to 8 hours. The frogs were gently blotted dry with tissues and reweighed. Their bladders were not drained with each reweighing so that the water balance response could be supressed naturally by the accumulation of bladder urine. The frogs usually did not urinate during the reweighing procedure if handled gently. If the weight of the frog decreased compared to a previous weighing indicating that urine had been voided, then that weight was discarded from the analyses and further data were not collected. After the final reweighing, the bladder was drained and the frog reweighed to determine the volume (mass) of urine.

Blood and urine were collected from a sample of control, hydrated frogs (kept in moist sand) and the experimental frogs after dehydration/rehydration. The frogs were pithed, the ventricle exposed, incised with sharp scissors, and a blood sample collected directly into a heparinised capillary tube. The blood was centrifuged and the plasma separated and frozen for subsequent analysis. Urine samples were also frozen for subsequent analysis. Plasma and urine were analysed for Na$^+$ and K$^+$ using a Varian model 475 atomic absorption spectrophotometer. Cl$^-$ was analysed using a Buchler-Cotlove amperometric titrator. NH$_4^+$ and urea concentrations were determined using a modified urease/alkaline hypochlorite/phenylnitroprusside spectrophotometric assay (Fawcett & Scott 1960). Osmotic concentration was determined using a Gonotek Osmomat freezing point osmometer.

Results

Initial body mass of the experimental turtle frogs was 7.46 ± 0.42 grams (n = 7), and dehydrated mass was 6.14 ± 0.37 grams (means values ± se); the dehydrated mass was 82.3 ± 0.80 % of the initial mass. The turtle frogs slowly rehydrated when placed in distilled water, reaching initial body mass after about 160 minutes and about 114% of initial mass after 450 minutes (Fig 1).

The instantaneous rate of rehydration, calculated from the weight gain between consecutive weighings, declined from the initially highest rate of 116 ± 14 mg g^{-1} h^{-1} to 19 ± 2 mg g^{-1} h^{-1} after 450 minutes (Fig 2). The average rate of weight gain, calculated from the accumulated weight increase from the beginning of the experiment, also declined during rehydration from 116 mg g^{-1} h^{-1} for the first reweighing period, to 51 mg g^{-1} h^{-1} after 450 minutes.

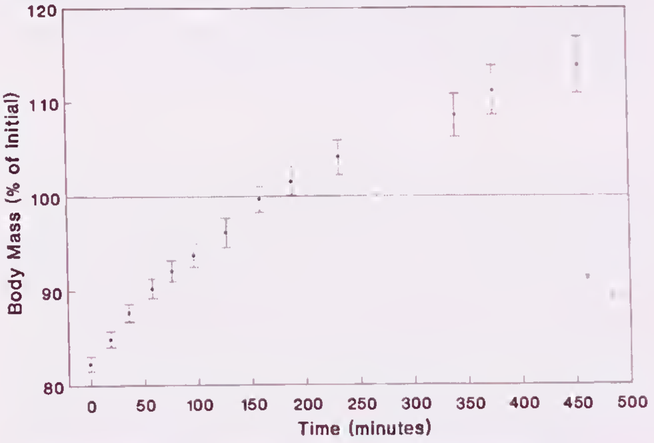

Figure 1 Increase in body mass of dehydrated turtle frogs *Myobatrachus gouldii*, after immersion in distilled water. Values are mean ± standard error, with the number of observations indicated.

The rate of rehydration would be expected to be proportional to the osmotic concentration difference between the body fluids and the ambient water (Shoemaker 1964). This osmotic concentration difference should decline during rehydration as the body fluids are diluted by reabsorbed water, and so the instantaneous rehydration rate should decline during rehydration. The effect of dehydration on body fluid osmotic concentration can be estimated from the weight loss. If we assume that the initial water content of the turtle frogs is 80 % (see Main & Bentley 1964, Bentley *et al.* 1958 for body water content of other Australian frogs), then dehydration to 82.3% of initial weight would increase the body fluid osmotic concentration 1.28 times. Similarly, the body fluid osmotic concentration can be estimated from the body mass at any stage of rehydration. Figure 2 shows that the predicted decline in instantaneous rate of water uptake (calculated from the initial instantaneous rate of water uptake and the estimated osmotic gradient between body fluids and ambient distilled water during rehydration) does not decrease by as much as the actual rate of water uptake. Similarly, the predicted instantaneous rehydration rate calculated from the final observed rehydration rate (at 450 min) and the estimated osmotic concentration difference during rehydration does not decrease by as much as the actual rate of water uptake.

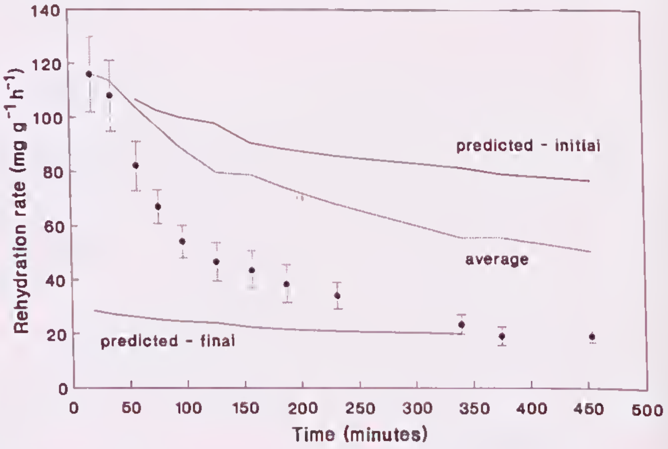

Figure 2. Instantaneous rehydration rate (solid circles; mean ± se, n) for dehydrated turtle frogs *Myobatrachus gouldii* immersed in distilled water. The average rehydration rate, calculated from the initial mass at time = 0, is also shown, along with the predicted rehydration rates (see text).

The average rehydration rate over the first 60 min is 31.1 mg cm^{-2} h^{-1} when expressed per calculated body surface area (cm^2 = 6 grams$^{2/3}$; Bentley *et al.* 1958, Main & Bentley 1964). The ventral skin of *M. gouldii* is smooth, and so the calculated surface area was not corrected for granularity.

The urine volume of rehydrated turtle frogs ranged from 5.0 to 22.6% of body mass; the three highest values (15.7, 20.1 and 22.6%) suggest a maximum bladder urine content of about 20% body mass.

The urine of hydrated, control frogs was significantly more dilute than plasma (Table 1). The Na$^+$, K$^+$ and Cl$^-$ concentrations were significantly lower for urine than plasma; there was no significant difference for NH$_4^+$; the urine concentration of urea was significantly greater than for plasma. There was no significant difference in osmotic or solute concentrations of plasma for dehydrated/rehydrated turtle frogs when compared to control frogs, except that plasma

Table 1

Osmotic and solute concentrations of plasma and urine for normally-hydrated turtle frogs *Myobatrachus gouldii* maintained in moist soil. Values are mean ± standard error with the number of observations in parentheses. The significance determined by t-test for the difference between plasma and urine values is indicated; ns = not significant, ** = $P<0.001$.

	Plasma	Urine
Osmotic Concentration (mOsm kg^{-1})	225 ± 8 (10)	24.7 ± 2.1 (10)**
Na^+ Concentration (mM)	111 ± 3 (10)	4.2 ± 0.5 (6)**
K^+ Concentration (mM)	4.1 ± 0.6 (14)	1.7 ± 0.2 (10)^{ns}
Cl^- Concentration (mM)	84.1 ± 3.3 (9)	2.9 ± 0.6 (11)**
NH_4^+ Concentration (mM)	2.0 ± 0.2 (9)	1.5 ± 0.2 (9)^{ns}
Urea Concentration (mM)	2.7 ± 0.4 (9)	12.7 ± 1.8 (9)**

urea concentration was higher (7.2 ± 0.7 mM, n=7, $P<0.005$) for the dehydrated/rehydrated frogs. The urine of dehydrated/rehydrated turtle frogs had a significantly higher osmotic concentration (47.2 ± 5.6 mOsm, n=6, $P<.005$), Na^+ concentration (10.7 ± 3.0 mM, n=6, $P<0.05$), K^+ concentration (3.2 ± 0.8 mM, n=7, $P<0.05$) and urea concentration (26.1 ± 4.2 mM, n=6, $P<0.01$) than for normal frogs.

Discussion

Amphibians absorb water across their skin when placed in fresh water, but the rate of cutaneous water influx varies with temperature, body size and species (Shoemaker & Nagy 1977, Shoemaker et al. 1992). Terrestrial amphibians tend to have high water influx rates to maximize the rate of rehydration when water is available, whereas aquatic species tend to have low water influx rates to minimise osmotic disturbance to the body fluid composition (Ewer 1952, Schmidt 1965, Bentley 1971, Shoemaker *et al.* 1992, Boutilier *et al.* 1992).

Turtle frogs, dehydrated to 82% of initial mass, rehydrated to initial body mass after about 160 min, and by 450 min had reached 114% of initial body mass (Fig 1). The overshoot in body mass might might indicate an inability of turtle frogs to maintain osmotic homeostasis in the unnatural situation of immersion in distilled water. However, rehydration after 450 min had not yet restored the normal plasma urea concentration, and the urine of dehydrated/rehydrated frogs still had higher osmotic, Na^+, K^+ and urea concentrations than urine of normal frogs.

The instantaneous rate of rehydration declined markedly from the initial high value of 116 mg g^{-1} h^{-1}, to 25 mg g^{-1} h^{-1} after 450 min (Fig 2). The instantaneous rate of rehydration did not decline with the predicted osmotic gradient for either the initial or final rehydration rate. This indicates that another non-osmotic control factor rapidly reduces the rehydration rate from the initial high value. The neurohypophyseal hormone AVT is one such potent regulator of cutaneous osmotic permeability and rehydration rate (Ewer 1952, Bentley 1971, Shoemaker *et al.* 1992).

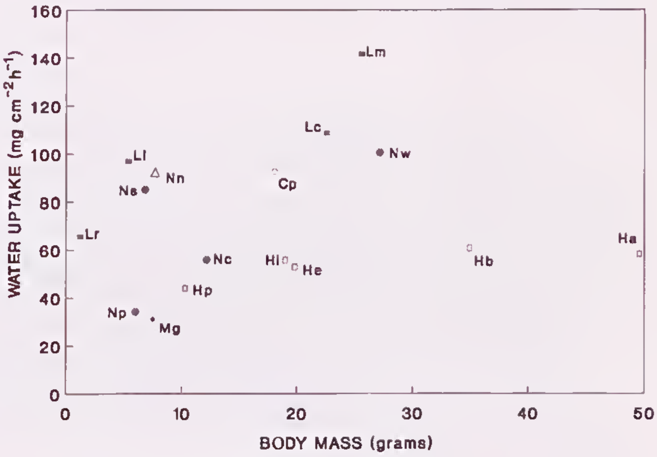

Figure 3. Comparison of rehydration rate (initial 60 min) for a variety of Western Australian amphibians, *Myobatrachus gouldii* (Mg, present study), *Neobatrachus pelobatoides* (Np), *N. centralis* (Nc), *N. wilsmorei* (Nw), *N. sutor* (Ns), *Heleioporus psammophilus* (Hp), *H. inornatus* (Hi), *H. eyrei* (He), *H. barycragus* (Hb), *H. albopunctatus* (Ha), *Notaden nichollsi* (Nn), *Cyclorana platycephala* (Cp), *Litoria rubella* (Lr), *L. latopalmata* (Ll), *L. caerulea* (Lc) and *L. moorei* (Lm); values from Bentley *et al.* (1958) and Main & Bentley (1964).

The average rehydration rate measured for *Myobatrachus gouldii* of 31.1 mg cm^{-2} h^{-1} (over the first 60 min of rehydration from 20% mass loss) is the lowest determined under comparable conditions for 15 species of Western Australian frogs from 4 genera (Bentley *et al.* 1958, Main & Bentley 1964; Fig 3). There is no obvious relationship between area-specific water uptake rate and body mass for these various Western Australian species. Bentley *et al.* (1958) reported a significant relationship between rehydration rate and environmental aridity for species of *Neobatrachus* (which dig shallow burrows in clay soil), but not for *Heleioporus* species (which dig deep burrows in sandy soil). Main & Bentley (1964) reported high rates of rehydration for some treefrogs (*Litoria moorei*, *L. caerulea* and *L. latopalmata*) and a desert burrowing frog, *Cyclorana platycephala*, which is closely related to tree frogs like *L. moorei* (Maxson *et al.* 1982, 1985). The lowest rehydration rates are for species which deeply burrow in sandy soils (*Heleioporus* spp, *Notaden nichollsi* and *Myobatrachus gouldii*); of these, only *Notaden* voluntarily enters water after metamorphosis to breed (Main 1968). The low rehydration rate of *Myobatrachus gouldii* presumably reflects the absence or rarity of free water in its natural habitat and lack of selective pressure for a marked water balance response.

The urinary bladder capacity of *M. gouldii* is about 20% body mass. This is greater than the bladder volume of aquatic frogs (about 1-5%) but is less than the extreme values of about 30-50% for some terrestrial, arid-adapted frogs (see

Shoemaker *et al.* 1992). The urine of *M. gouldii* is relatively dilute compared to plasma for both normally-hydrated frogs (Table 1) and dehydrated/rehydrated frogs. Consequently, bladder urine would be an important water store for *M. gouldii* during dry periods.

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